SIRE1 Retrotransposons in Barley (Hordeum vulgare L.)

B. Cakmak, S. Marakli, and N. Gozukirmizi

Department of Molecular Biology and Genetics, Istanbul University, Istanbul 34134, Turkey
e-mail: sevgi.marakli@istanbul.edu.tr

Received May 30, 2014

Abstract—Sireviruses are genera of copia LTR retrotransposons with a unique genome structure among retrotransposons. Barley (Hordeum vulgare L.) is an economically important plant. In this study, we used mature barley embryos, 10-day-old roots and 10-day-old leaves derived from the same barley plant to investigate SIRE1 retrotransposon movements by Inter-Retrotransposon Amplified Polymorphism (IRAP) technique. We found polymorphism rates between 0—64% among embryos, roots and leaves. Polymorphism rates were detected to be 0—27% among embryos, 8—60% among roots, and 11—50% among leaves. Polymorphisms were observed not only among the parts of different individuals, but also on the parts of the same plant (23—64%). The internal domains of SIRE1 (GAG, ENV and RT) were also analyzed in the embryos, roots and leaves. Analysis of band profiles showed no polymorphism for GAG, however, different band patterns were observed among samples for RT and ENV. The sequencing of SIRE1 GAG, ENV and RT domains revealed 79% similarity for GAG, 96% for ENV and 83% for RT to copia retrotransposons. Comparison between barley retrotransposons and SIRE1 in barley indicated that SIRE1-GAG, ENV and RT might be diverge earlier from barley retrotransposons. SIRE1 sequences were compared with SIRE1 in barley, results showed the closest homologues were SIRE1-ENV and SIRE1-RT sequences, and SIRE1-GAG sequences was a sister group to sequences of Glycine max. This study is the first detailed investigation of SIRE1 retrotransposon and its role in barley genome. The obtained findings are expected to contribute to the comprehension of SIRE1 retrotransposon and its role in barley genome.

DOI: 10.1134/S1022795415070029

INTRODUCTION

Retrotransposons are mobile genetic elements [1] that encode the proteins needed for their own replication and integration back into the genome [2]. Most of the characterized LTR retrotransposons belong to either Tyl-copia or Ty3-gypsy group [3]. Tyl-copia retrotransposons are abundant in plants, and are closely related to the retroviruses, but have a different polyprotein (pol) gene order [1].

Sireviruses are plant-specific LTR retrotransposons. Sireviruses are also unique among LTR retrotransposons in terms of their own genome structure [4, 5]. It is the only copia genus whose members often possess a putative envelope-like (ENV-like) gene [6]. Due to their host specificity they were originally termed Agroviruses [7], before being renamed to Sireviruses (derived from the SIRE1 element of soybean [8] by the International Committee on the Taxonomy of Viruses—ICTV) [9]. This has left as yet open questions on the position of Sireviruses within the LTR retrotransposon order, and whether they should actually be considered as viruses. Based on published work on Sireviruses [4, 5, 10—13], it is safe to assume that the characteristics of their life cycle correspond to that of typical LTR retrotransposons and not of viruses.

Several different retrotransposon-based markers have been developed to detect retrotransposition that results in insertion of retrotransposon copies and therefore generates polymorphism [14—17]. In the Inter-Retrotransposon Amplified Polymorphism (IRAP) technique, which produces retrotransposon-based markers, regions flanked by two LTR-retrotransposons or solo LTRs are amplified [18]. In this method, polymorphisms are detected by the presence or absence of the PCR product and lack of amplification indicates absence of the retrotransposon at a particular region [19]. IRAP has been used in studies on the genetic diversity in many species [16, 20—22].

The aim of this study was to identify SIRE1 retrotransposon in barley. For this purpose, SIRE1 retrotransposon insertion patterns and also SIRE1 internal domains (GAG, ENV and RT) were investigated in germinating barley seedling parts (roots and shoots separately). Embryos were used as control materials. Moreover, evolutionary relationships between other barley retrotransposon and SIRE1 sequences identified in this study, and between SIRE1 in other plants and SIRE1 in barley.

MATERIALS AND METHODS

Barley (Hordeum vulgare L. cv. Tokak 157/37) mature seeds were surface-sterilized in 20% commer-
were mixed with formed by using a thermal cycler (Bio-Rad, obtained from Chesnay et al. [24]. PCR was per-
was 5’-CAGTTATGCAAGTGGGATCAGCA-3’ as

GeneRuler™ marker (1 mmol/L EDTA, pH 8.0). A molecular weight
TAE buffer (90 mmol/L Tris, 20 mmol/L acetic acid,
72°C for 10 days in a programmed growth chamber
16 hours. Then, embryos of those seeds were dissected and used as control materials. DNA was isolated from four embryos, four roots and four leaves according to Kidwell and Osborn [23]. Spectrophotometric analyses were conducted to determine the quantity and quality of the isolated DNA. DNA concentrations were equalized. The amplification of SIRE1 by the IRAP method and SIRE1 internal sequences were carried out.

IRAP Analysis

SIRE1 movements were investigated by the IRAP technique. The primer sequence used for IRAP was 5’–CAGTTATGCAAGTGGGATCAGCA–3’ as obtained from Chesnay et al. [24]. PCR was performed by using a thermal cycler (Bio-Rad, T100™) in a total volume of 20 µL, containing 3.5 µL of sterile distilled water, 0.5 µL of 10 mmol/µL dNTP mixture (0.25 mM/µL), 4 µL of primer (1 µM/µL), 2 µL of 10 ng/µL template genomic DNA and 10 µL of 2x Saphire enzyme mix (Takara, RR350A). The values given in parentheses were the final concentrations. PCR conditions were as follows: initial denaturation at 94°C (3 min) followed by 30 cycles of denaturation at 94°C (20 s), annealing at 47°C (20 s) and extension at 72°C (2 min). The reaction was completed by additional extension at 72°C for 10 min.

Twenty-microliter aliquots of IRAP-PCR products were mixed with 4 µL 6× loading buffer (10 mmol/L Tris–HCl, 60 mmol/L EDTA, pH 8.0, 0.3% bromophenol blue, 60% glycerol) and resolved in 2.5% agarose gel electrophoresis at 100 V for 120 min in 1× TAE buffer (90 mmol/L Tris, 20 mmol/L acetic acid, 1 mmol/L EDTA, pH 8.0). A molecular weight marker (GeneRuler™ DNA Ladder Mix, SM0331. Thermo Scientific) was also loaded to determine the sizes of the amplicons. After running, the gels were photographed on a UV transilluminator. The IRAP bands were scored visually.

Data Analysis

Well-resolved bands were scored as a binary value, “1” for presence and “0” for absence. The binary matrix (1/0) was used to calculate the similarity by Jaccard’s coefficient [25] among embryos, 10-day-old roots and 10-day-old leaves.

Due to the dominant nature of IRAP [26], the Jaccard’s coefficient, one of the appropriate indices for dominant markers, was chosen. The Jaccard’s similarity index was calculated using the formula: \( \frac{NB}{(NB + NA)} \), where \( NB \) is the number of bands shared by 2 samples, \( NA \) indicates amplified fragments in sample A, and \( NB \) represents amplified fragments in sample B.

SIRE1-LTR Analysis

In addition to IRAP analysis, primers of SIRE1-LTR sequences were obtained from Laten et al. [8] (Table 1, primer nos. 7–8). PCR was performed in a total volume of 20 µL, containing 7.8 µL of sterile distilled water, 2 µL of 10× buffer (1×), 2 µL of 25 mmol/L MgCl₂, (2.5 mmol/L), 2 µL of 2 mmol/L dNTP mixture (0.2 mmol/L), 7.8 µL of each primer (0.5 µM/µL), 4 µL of 10 ng/µL template genomic DNA (2 ng/µL), and 0.2 µL of 5 U/µL High Fidelity PCR Enzyme Mix (K0192, Thermo Scientific). The values given in parentheses were the final concentrations. PCR conditions were as follows: initial denaturation at 95°C (3 min) followed by 30 cycles of denaturation at 94°C (30 s), annealing at 39°C (30 s) and extension at 72°C (2 min). The reaction was completed by an additional extension at 72°C for 10 min. Twenty microliters of PCR products were mixed with 4 µL 6× loading buffer and were resolved in an agarose gel (2.5% concentration) at 75 V for 45 min in 1× TAE buffer. A molecular weight marker (GeneRuler™ DNA Ladder Mix,

<table>
<thead>
<tr>
<th>No.</th>
<th>Primer</th>
<th>Sequence (5’ → 3’)</th>
<th>( T_a, ^°C )</th>
<th>Target size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SIRE1-ENV/F</td>
<td>ACATTTGCTCGACACAGGC</td>
<td>49</td>
<td>2000</td>
</tr>
<tr>
<td>2</td>
<td>SIRE1-LTR/R</td>
<td>ATATTTCCGGCAGATG</td>
<td>51</td>
<td>1300</td>
</tr>
<tr>
<td>3</td>
<td>SIRE1-LTR/F</td>
<td>TGGAAAGTTTGAAAACGTGGC</td>
<td>40</td>
<td>900</td>
</tr>
<tr>
<td>4</td>
<td>SIRE1-GAG/R</td>
<td>AGTCGAAAAAGGGATGTCCG</td>
<td>39</td>
<td>1000</td>
</tr>
<tr>
<td>5</td>
<td>SIRE1-RT/F</td>
<td>GAGGCACGTGACTGATGATTC</td>
<td>49</td>
<td>2000</td>
</tr>
<tr>
<td>6</td>
<td>SIRE1-RT/R</td>
<td>TTCTTTGCATACTTGTGGAG</td>
<td>51</td>
<td>1300</td>
</tr>
<tr>
<td>7</td>
<td>SIRE1-LTR/F</td>
<td>TGGAAAGTTTGAAAACGTGGC</td>
<td>40</td>
<td>900</td>
</tr>
<tr>
<td>8</td>
<td>SIRE1-LTR/R</td>
<td>ATATTTCCGGCAGATG</td>
<td>39</td>
<td>1000</td>
</tr>
</tbody>
</table>

RUSSIAN JOURNAL OF GENETICS Vol. 51 No. 7 2015